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RESPONSES OF TROUT FRY HELD IN SEALED POLYETHYLENE BAGS

by

Patrick F. Chapman
Fish Hatchery Superindendent I McCall Hatchery

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ABSTRACT

Tests were conducted at McCall Hatchery with rainbow (*Salmo gairdneri*) and cutthroat trout (*Salmo clarki*) fry to determine proper loading densities when held in polyethylene bags for six hours. Rainbow trout at all sizes tested could be held successfully at a density of no more than 1 lb/1.2 gal. Cutthroat trout between 917.7 and 1198.6/lb could be held successfully at a density of only 0.75 lb/1.2 gal, while cutthroat at 488.5/lb performed satisfactorily when held at no more than 1 lb/1.2 gal. Higher metabolic rates and faster use of available oxygen was determined to be the cause for the poor performance of cutthroat trout as compared with larger rainbow trout in early tests.

Author:

Patrick F. Chapman
Fish Hatchery Superintendent I

INTRODUCTION

During the summer of 1981, McCall Hatchery personnel aerially stocked mountain lakes with rainbow. trout (Salmo *gairdneri*) and rainbow trout x cutthroat trout (*Salmo* *clarki*) hybrid fry. For each lake to be stocked, a maximum of one pound of fry was loaded in a three-gallon polyethylene milk bag containing slightly more than one gallon of chilled water and then each bag was inflated with oxygen and sealed. Prior to stocking, mortality of fry in many bags exceeded 50% for reasons not fully understood (Chapman 1981). Infection of the hybrids by various bacteria and parasites undoubtedly contributed to the high mortality experienced by these fish; however, the rainbows sometimes experienced similar levels of mortality, even though these fish were **in** apparently good health. Loading trout fry at these densities did not result in excessive mortality in the past at McCall Hatchery (Bill Doerr personal communication, Hagerman, Idaho) or at Mackay Hatchery (Bob Vaughn personal communication, Mackay, Idaho).

Again in the summer of 1982, McCall. Hatchery personnel were to aerially stock mountain lakes with rainbow trout and cutthroat trout fry using the same methods as in the past. Little mortality of fry was anticipated between bag loading and stocking, since the health of both species was excellent. However, the first mountain lake stocking flight revealed mortalities of up to approximately 50% in bags loaded with one pound of fry. Since the cutthroat trout, in general, were experiencing higher mortalities than the rainbow trout, it was felt that some species-specific cause of mortality might be involved.

In an effort to determine loading densities for rainbow and cutthroat trout fry resulting in minimal mortality and stress over time periods similar to those experienced when aerially stocking mountain lakes, bag-loading tests were immediately begun. These tests were designed to imitate, as closely as possible, conditions experienced and procedures used when loading fish in bags and stocking mountain lakes by plane. This report describes these tests and the results, as well as the results of actual field application of these findings.

OBJECTIVES

1. Determine maximum amount of rainbow and cutthroat trout fry that can be loaded in three-gallon polyethylene bags while maintaining acceptable levels of mortality and stress over periods of time similar to those experienced when stocking mountain lakes.

2. Apply these findings to actual mountain lake stocking operations to achieve optimum survival of fish while in polyethylene bags.

3. Determine the cause of higher rates of mortality experienced by cutthroat trout when loaded in three-gallon polyethylene bags at similar densities and conditions as rainbow trout.

TECHNIQUES USED

Fish used in the tests were rainbow trout originating from eggs purchased from Aqua-Life Corporation (Buhl, Idaho), and cutthroat trout originating from eggs taken at Henrys Lake State Fish Hatchery (Mack's Inn, Idaho). Eggs of both species were received at McCall Summer Chinook Salmon Hatchery (McCall, Idaho) while in the eyed stage, and were incubated and hatched in Heath vertical-stack incubators (Heath Tecna Corporation, Kent, Washington). Following button-up, the fry were reared in concrete vats with water flows sufficient to exceed three water exchanges per hour. Fry were fed various commercially-prepared diets on an hourly basis. Fry were withheld feed for 36 hours prior to beginning each test. Pound counts were performed using standard techniques the day before each test to determine average size of the fish.

Twelve tests were conducted between 29 July and 23 September 1982, using various combinations of loading densities, water temperatures, and fish size for both species tested (Table 1). In addition, three tests without fish were conducted to serve as controls.

For each test, eight three-gallon polyethylene double-walled milk bags manufactured by B-Bar-B, Inc. (New Albany, Indiana) were loaded with water, fish, and oxygen. In tests using chilled water, ice was added to water contained in an 18-gallon washtub to maintain water temperatures between 3 and 5 C. Water temperatures were determined in all tests with a YSI Model 57 dissolved oxygen meter (Yellow Springs Instrument Company, Yellow Springs, Ohio). Water used in all tests was obtained from the head of whichever vat contained the fish tested. In chilled-water tests, a measured amount of water was added to the bags by pouring it over ice contained in a funnel. In unchilled-water tests, water was obtained directly from the head of the vat and poured into each bag using the funnel without ice.

Table 1. Tests conducted with rainbow and cutthroat trout using varying size fish, pounds of fish and amounts of water and oxygen in polyethylene bags.

Test Number	Species Tested	Number Per Pound	Pounds In Bag	Gallons Water	Amount (seconds) 1/ Oxygen Added
1	Rainbow	560.7	1.0	1.2	10
2	Rainbow	560.7	1.5	1.2	10
3	Rainbow	272.2	1.0	1.2	10
4	Rainbow	272.2	1.25	1.2	10
5	Cutthroat	1198.6	1.0	1.2	10
6	Cutthroat	1198.6	0.75	1.2	10
7	Cutthroat	948.7	1.0	1.2	10
8	Cutthroat	917.7	1.0	1.2	10
9	Cutthroat	917.7	1.0	1.2 2/	10
10	Cutthroat	488.5	1.0	1.2	10
11	Cutthroat	488.5	1.25	1.2	10
12	Cutthroat	488.5	0.75	0.5 3/	12
13	Control	-	-	1.2	10
14	Control		-	1.2 2/	10
15	Control	-	-	0.5	12

1/ Oxygen injected at delivery pressure of 75 psi.
2/ Water not chilled with ice prior to loading.
3/ 60% of water loaded in bag chilled with ice.

When a bag was filled with the correct amount of water, fry were netted from the vat, weighed in a coffee can containing 740 ml water on an Ohaus triple-beam balance (Ohaus Scale Corporation, Florham Park, NJ), and loaded into the bag.

Each bag was immediately injected with oxygen at 75 psi delivery pressure using an acetylene cutting torch adapted with rubber tubing terminating in a milk bag cap. Oxygen was added to each bag until pressure in the bag felt similar to that of bags loaded for actual mountain lakes stocking flights, or approximately ten and twelve seconds of delivery time for bags containing 1.2 and 0.5 gallons of water, respectively. After completion of oxygen injection, the bag was sealed with the cap supplied with each bag.

Upon sealing each bag, the time was recorded and the bag was placed on the floor of the hatchery building in a flat position. Room temperatures during the study ranged between 14 and 18 C. Bags were not disturbed until they were checked at the conclusion of the test. No attempt was made to mix the oxygen in the water with agitation.

Dissolved oxygen content and water temperature was measured with the YST dissolved oxygen probe and number of mortalities noted in the first bag loaded for each test immediately after adding fish (prior to injection with oxygen), and one-half hour after sealing. Subsequent bags were opened in the sequence loaded and the same parameters measured at each half-hour interval up to two hours elapsed time and hourly thereafter up to an elapsed time of six hours (Table 2).

Methods used in control tests were the same as other tests, except no fish were added to these bags.

Records of water temperatures (obtained with a hand-held mercury thermometer) and fish mortality just prior to stocking were kept during mountain lake stocking flights.

Due to physical and time constraints involved in stocking the lakes by plane, it was not possible to obtain dissolved oxygen readings.

Table 2. Schedule for measuring dissolved oxygen, water temperature, and mortality in polyethylene bags.

Bag number	Hours sealed prior to measurements	Bag number	Hours sealed prior to measurements
1	0	4	2.0
1	0.5	5	3.0
2	1.0	6	4.0
3	1.5	7	5.0
		8	6.0

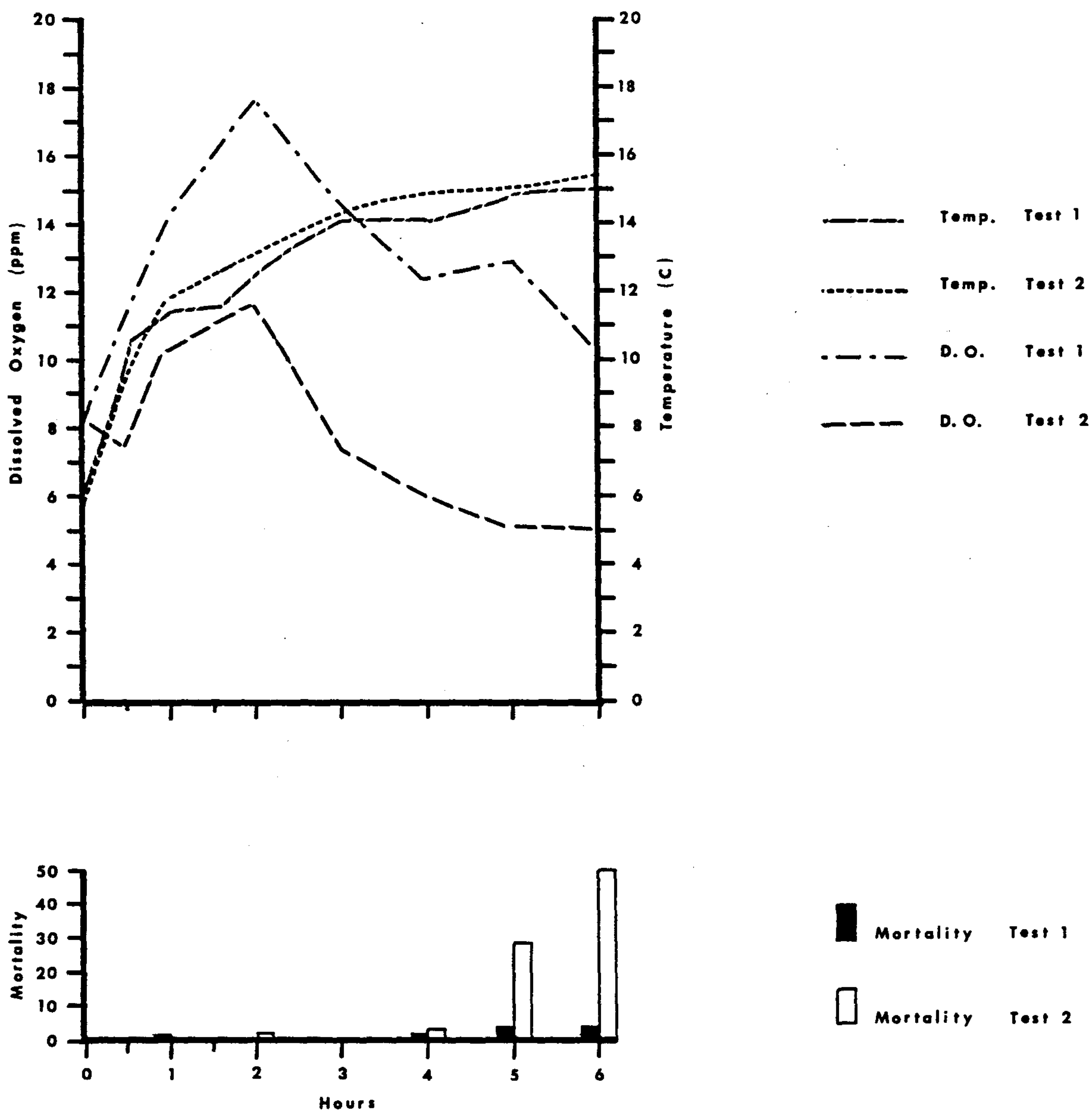
FINDINGS

Initial tests with rainbow and cutthroat trout fry revealed a marked difference in the performance of a species when loaded in polyethylene bags at different densities and a marked difference between the performance of both species when loaded at similar densities. Tests 1 and 2 with rainbow trout at 560.7/lb indicated satisfactory rates of oxygen consumption and mortality when this species was loaded at a density of 1 lb/1.2 gal, but unsatisfactory rates of oxygen consumption and mortality when loaded at a density of 1/5 lb/1.2 gal (Fig. 1). Cutthroat trout at 1,198.6/lb in Test 5 exhibited a different response to a loading density of 1 lb/1.2 gal than did rainbow trout (Fig. 2). Cutthroat loaded at this density suffered unsatisfactory levels of mortality and oxygen content measured in the bags was low throughout the test. In contrast, cutthroat trout in Test 6 at 1,198.6/lb loaded at a density of 0.75 lb/1.2 gal experienced little mortality and oxygen content in the bags was adequate throughout the test (Fig. 2).

Test 7 represents a duplicate of Test 5 using somewhat larger cutthroat trout fry (948.7/lb) than Test 5 (1,198.6/lb). Cutthroat trout in this test (Fig. 3) exhibited a much better response to a loading density of 1 lb/1.2 gal than did the smaller cutthroat in Test 5. Mortality was nearly nonexistent in Test 7, while oxygen content in the bags exhibited much higher levels than in Test 5.

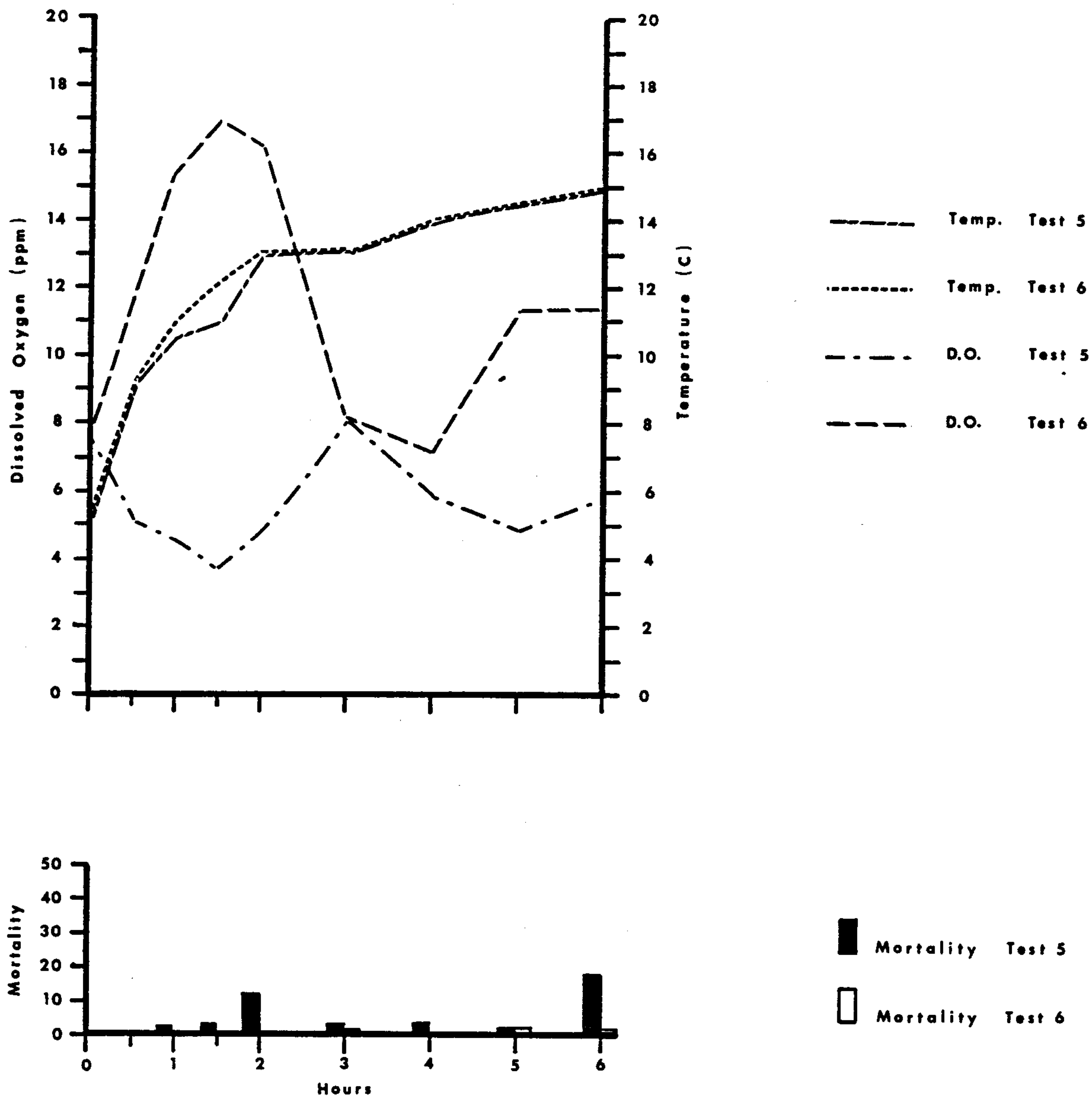
Tests 8 and 9 compared the performance of cutthroat trout at 917.7/lb when loaded at densities of 1 lb/1.2 gal into bags containing chilled and unchilled water (Fig. 4). Oxygen content remained consistently and substantially lower in the unchilled water as compared to the chilled water, and mortality of fish in the bags containing unchilled water was somewhat greater than that in chilled water bags.

Immediately following these tests, mountain lake stocking flights were continued, using rainbow and cutthroat trout fry of various sizes and at three different loading densities. Flight 1 revealed unacceptable levels of mortality of cutthroat trout (848.2/lb) in bags loaded at a density of 1 lb/1.2 gal, but acceptable survival in bags loaded at densities of 0.75 lb/1.2 gal (Table 3). Rainbow trout averaging 453/lb experienced negligible mortality when loaded in bags at a density of 1 lb/1.2 gal. Flights 2 and 3 had excellent and acceptable levels of mortality, respectively, in both species.



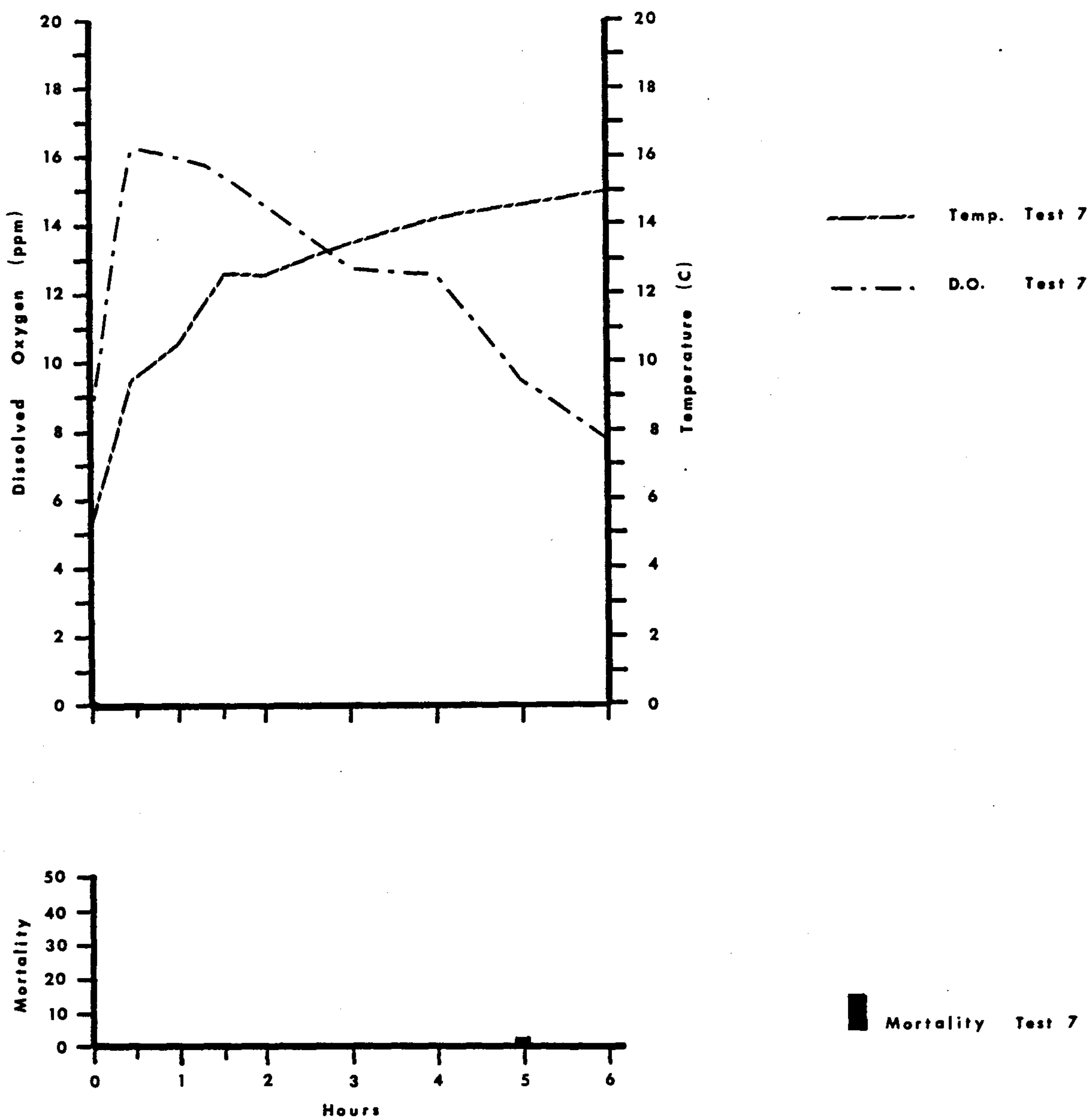
Test Number	Species Tested	Number Per Pound	Pounds In Bag	Gallons Water	Amount (seconds) Oxygen Added
1	Rainbow	560.7	1.0	1.2	10
2	Rainbow	560.7	1.5	1.2	10

Figure 1. Mortality, dissolved oxygen consumption and temperature change rates for tests 1 and 2 of rainbow trout fry loaded in polyethylene bags.



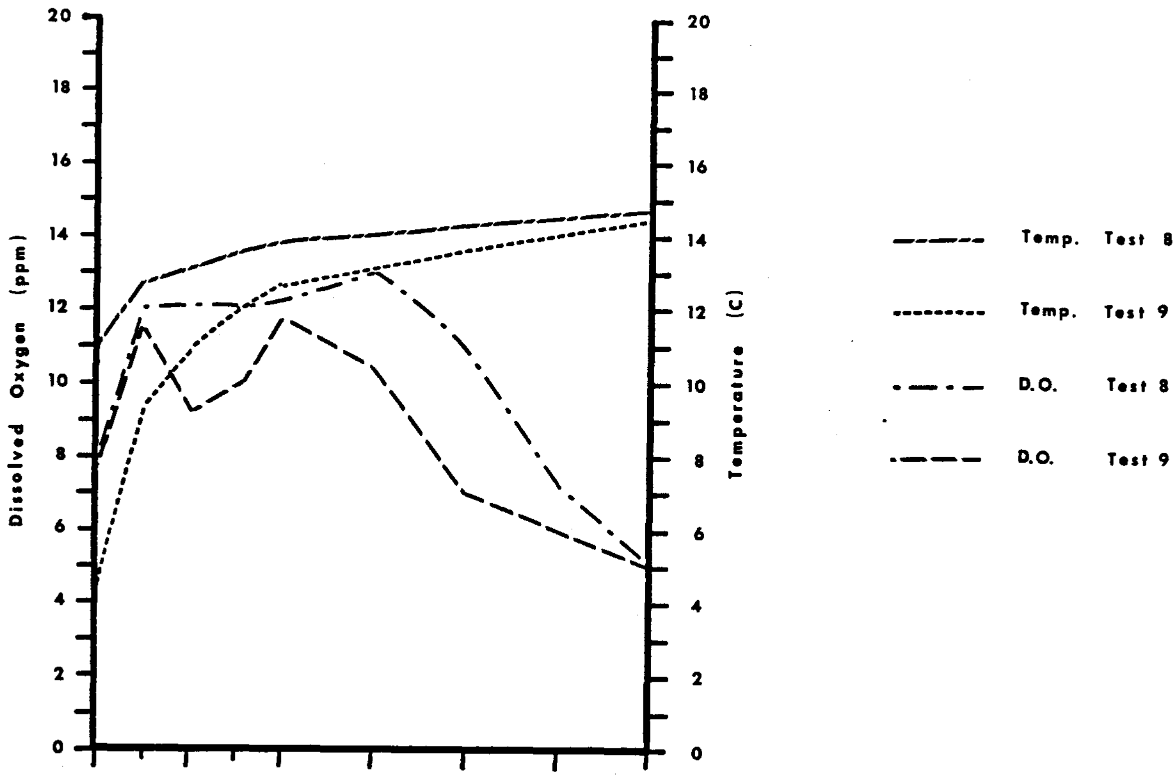
Test Number	Species Tested	Number Per Pound	Pounds In Bag	Gallons Water	Amount (seconds) Oxygen Added
5	Cutthroat	1198.6	1.0	1.2	10
6	Cutthroat	1198.6	0.75	1.2	10

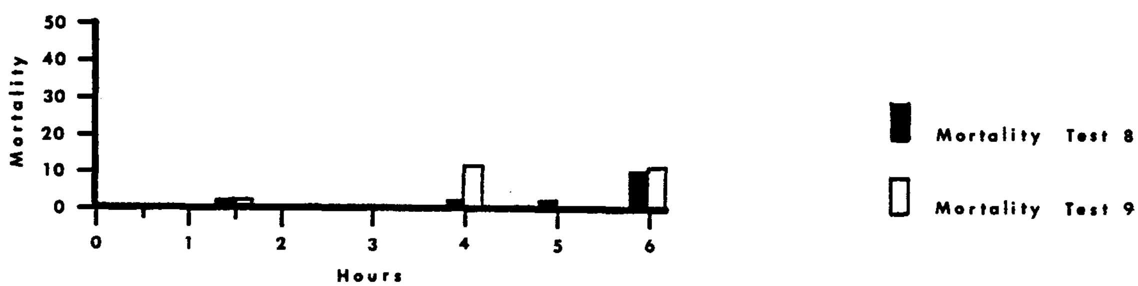
Figure 2. Mortality, dissolved oxygen consumption and temperature change rates for tests 5 and 6 of cutthroat trout fry loaded in polyethylene bags.



Test Number	Species Tested	Number Per Pound	Pounds In Bag	Gallons Water	Amount (seconds) Oxygen Added
7	Cutthroat	948.7	1.0	1.2	10

Figure 3. Mortality, dissolved oxygen consumption and temperature change rates for test 7 of cutthroat trout fry loaded in polyethylene bags.





Test Number	Species Tested	Number Per Pound	Pounds In Bag	Gallons Water	Amount (seconds) Oxygen Added
8	Cutthroat	917.7	1.0	1.2	10
9	Cutthroat	917.7	1.0	1.2 (non-iced)	10

Figure 4. Mortality, dissolved oxygen consumption and temperature change rates for tests 8 and 9 of cutthroat trout fry loaded in polyethylene bags.

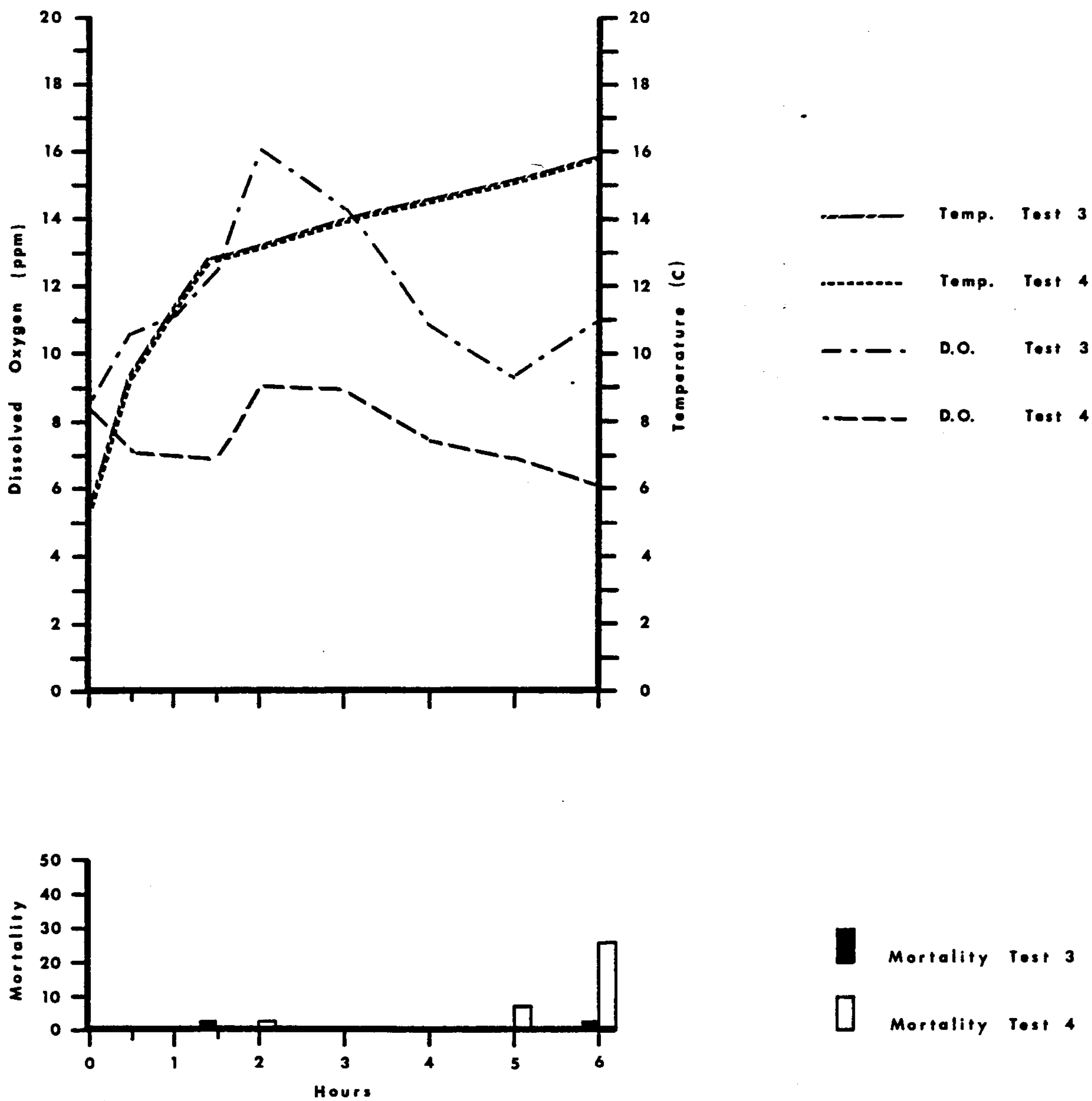
Tests 3, 4, 10, and 11 tested the response of rainbow and cutthroat trout roughly twice the size of fish in earlier trials. Tests 3-and 4 revealed that rainbow trout averaging 272.2/lb suffered lower levels of mortality and consumed less oxygen when loaded at densities of 1 lb/1.2 gal than did those loaded at 1/25 lb/1.2 gal (Fig. 5). Tests 10 and 11 using cutthroat trout averaging 488.5/lb exhibited an even more dramatic difference in the performance of those loaded at 1 lb/1.2 gal, and of those loaded at 1.25 lb/1.2 gal (Fig. 6). Mortality and oxygen consumption were much greater at the higher loading density and were at unacceptable levels.

Test 12 was conducted to investigate the response of cutthroat trout when placed in bags containing only 0.5 gal water, while Test 15 was a control for this trial. Cutthroat trout at 488.5/lb in Test 12 loaded at 0.75 lb/0.5 gal experienced no mortality and had much higher levels of dissolved oxygen in the bags in this trial than any other trial (Fig. 7). The control test, Test 15, investigated the rate of oxygen uptake in 0.5 gal water and revealed a rapid increase in dissolved oxygen content of the water to a point greater than 20 ppm for the duration of the trial, and therefore, unmeasurable with the equipment utilized in this study (Fig. 7).

Tests 13 and 14 also were control tests, but compared the difference in oxygen uptake of 1.2 gal of chilled and unchilled water. The chilled water in Test 13 took up oxygen at a slightly greater rate than did the unchilled water in Test 14 (Fig. 8); however, both reached dissolved oxygen contents of greater than 20 ppm, although at slightly slower rates than Test 15.

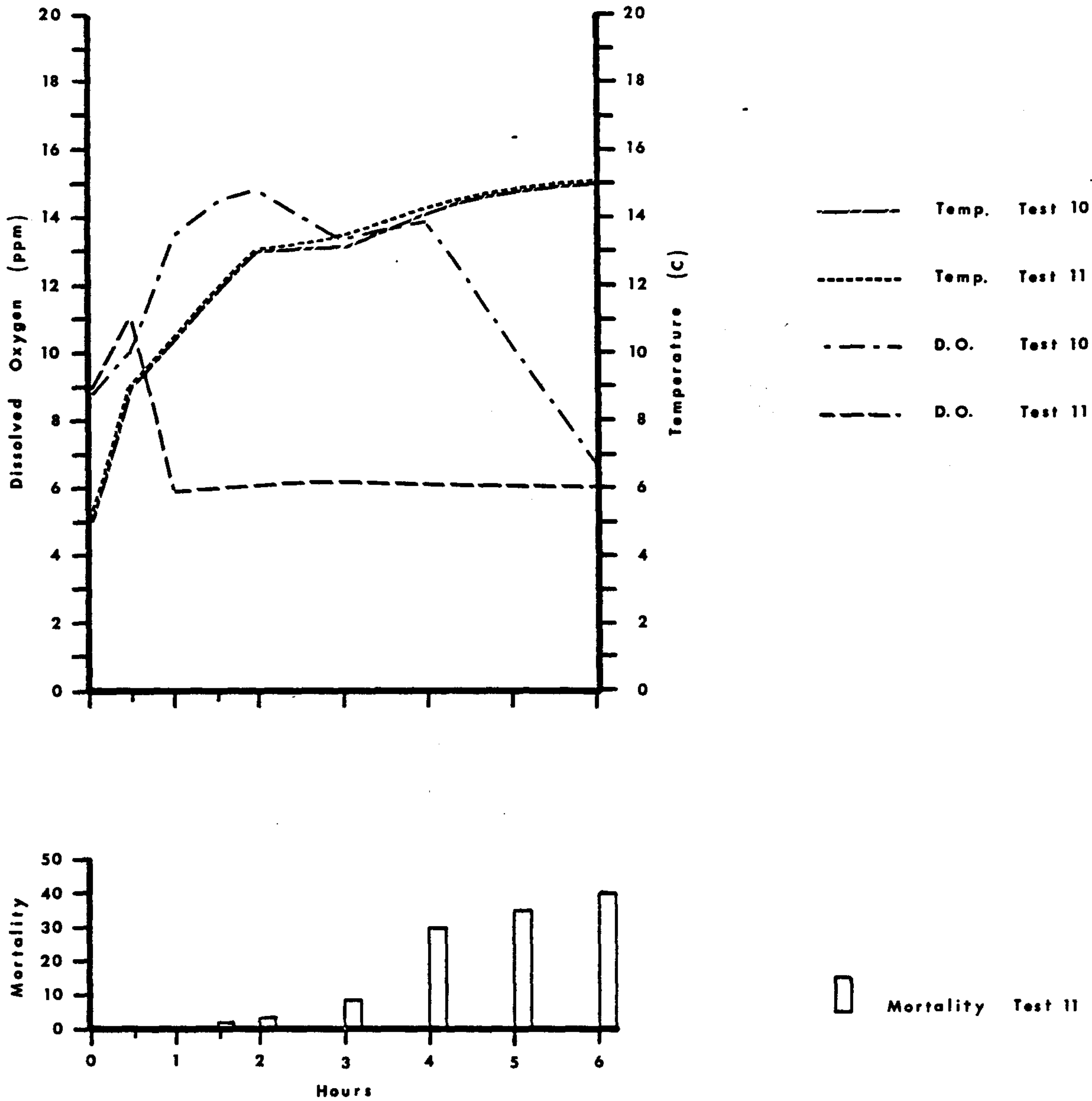
DISCUSSION

Tests 1, 2, 5, and 6 (Fig. 1 and 2) reinforced the results of early mountain lake stocking flights by showing that, with the bag loading methods used in past operations, no more than one lb of rainbow trout fry could be successfully held in bags for up to six hours,while cutthroat trout could only survive well at densities no greater than 0.75 lb per bag. Densities of fish at levels greater than these resulted in undesirable levels of dissolved oxygen in the water and high levels of mortality. Tests 7 and 8 (Fig. 3 and 4), however, indicated good survival and dissolved oxygen content of the water when cutthroat of somewhat larger size were held at densities greater than those indicated as maximum for cutthroat trout in earlier tests.



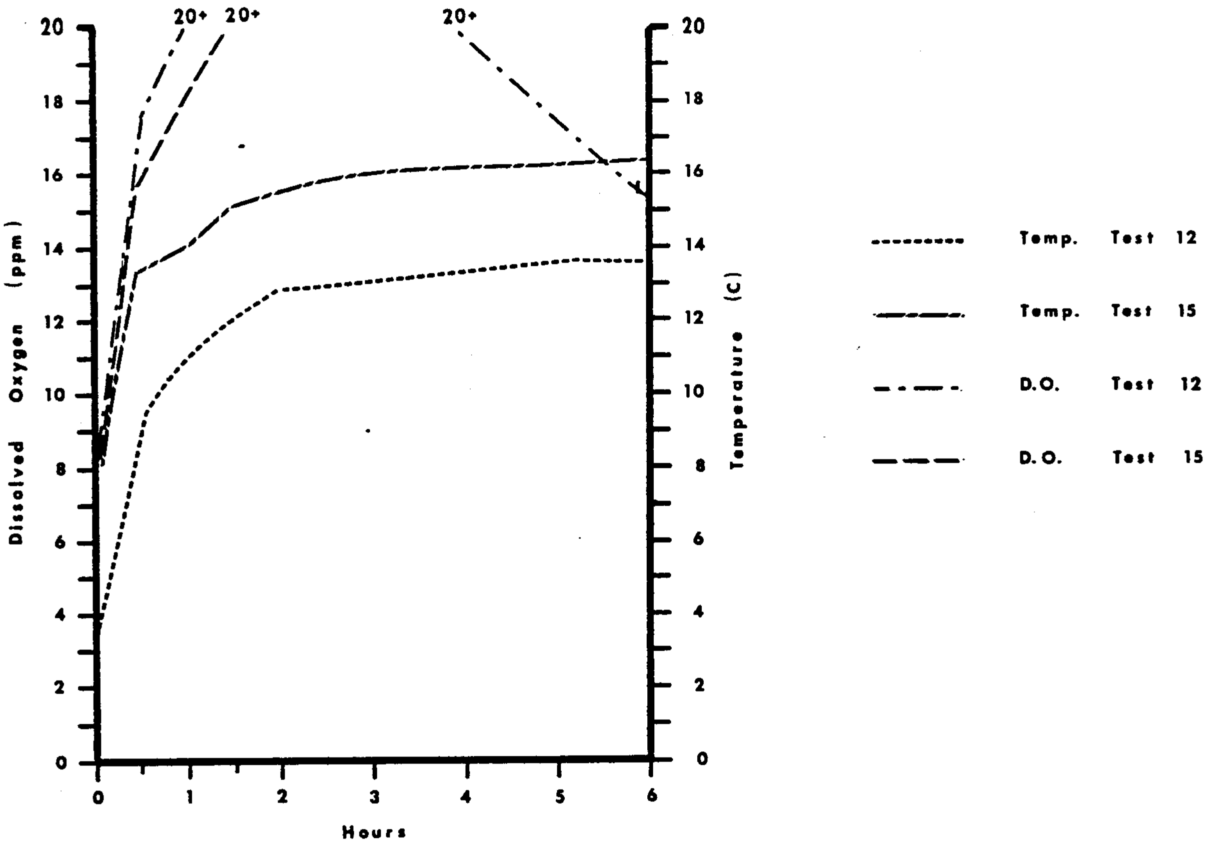
Test Number	Species Tested	Number Per Pound	Pounds In Bag	Gallons Water	Amount (seconds) Oxygen Added
3	Rainbow	272.2	1.0	1.2	10
4	Rainbow	272.2	1.25	1.2	10

Figure 5. Mortality, dissolved oxygen consumption and temperature change rates for tests 3 and 4 of rainbow trout fry loaded in polyethylene bags.



Test Number	Species Tested	Number Per Pound	Pounds In Bag	Gallons Water	Amount (seconds) Oxygen Added
10	Cutthroat	488.5	1.0	1.2	10
11	Cutthroat	488.5	1.25	1.2	10

Figure 6. Mortality, dissolved oxygen consumption and temperature change rates for tests 10 and 11 of cutthroat trout fry loaded in polyethylene bags.



Test Number	Species Tested	Number Per Pound	Pounds In Bag	Gallons Water	Amount (seconds) Oxygen Added
12 1/	Cutthroat	488.5	0.75	0.5 2/	12
15	Control	-	-	0.5	12

1/ No mortality in test 12.
2/ 60% of water chilled with ice when loading bags.

Figure 7. Dissolved oxygen consumption and temperature change rates for test 12 of cutthroat trout loaded in polyethylene bags and control test 15.

The data obtained from Tests 7 and 8 led to the belief that perhaps cutthroat trout could indeed be held at greater densities than indicated by earlier tests and so the next mountain lake stocking flight consisted of bags of fish loaded at densities resulting in good survival during initial tests. As Table 3 reveals, rainbow trout performed well at a density of 1 lb/1.2 gal, but cutthroat trout performed very poorly at this density. When held at a density of 0.75 lb/1.2 gal, cutthroat exhibited good survival.

Based on these results, all subsequent mountain lake stocking flights were conducted using rainbow trout loaded in bags at 1 lb/1.2 gal and cutthroat trout loaded at 0.75 lb/1.2 bal. Mortality of both species during these flights was usually nonexistent and rarely exceeded five fish per bag.

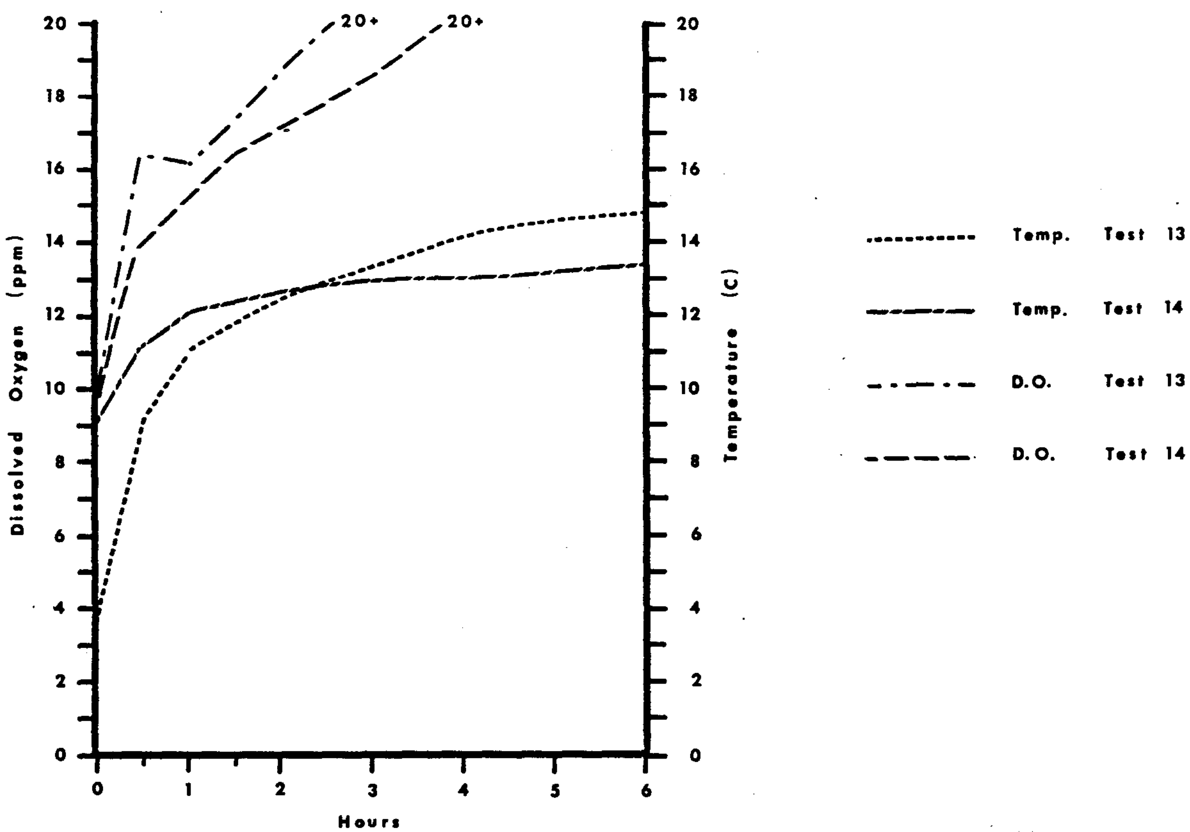
That mortality of cutthroat trout loaded at 1 lb/1.2 gal during mountain lake stocking flights was much greater than in tests under similar conditions is significant because it indicates that test conditions did not sufficiently imitate actual stocking flight conditions. It is obvious from these results that the handling of the bags and other operations involved in stocking mountain lakes is much more stressful to trout fry, and cutthroat trout fry in particular, than the operations that occurred during the tests. This is important because it reveals the need to maintain the highest levels of dissolved oxygen possible in the water that trout fry are held in prior to stocking in an effort to compensate for these stressful conditions.

After the initial mountain lake stocking flights and prior to initiation of these studies, concern was expressed that the cause of the high mortalities observed during these flights might be temperature shock resulting from loading fish into water chilled 7-9 C below rearing water temperatures. The fact that, in all tests, mortalities did not generally occur until dissolved oxygen content reached low levels and that mortality in Test 9 involving unchilled water (Fig. 4) was actually higher than a similar test with chilled water tends to disprove this argument. Loading fish in chilled water, in fact, was beneficial because this method resulted in higher levels of dissolved oxygen throughout the test than when loaded in unchilled water. This result probably is due to the fact that water of lower temperature has a greater affinity for oxygen and uptakes oxygen at a faster rate than water of higher temperature, as is demonstrated in Tests 13 and 14 (Fig. 8), and by the fact that fish in colder water have lower rates of metabolism, and therefore require less oxygen than fish in warmer water.

Table 3. Performance of rainbow and cutthroat trout fry of various sizes contained in polyethylene bags during mountain lake stocking flights.

Flight	Species	Number Per Pound	Pounds In Bag 1/	Total Fish Bagged	Approximate Mortality At Stocking	Maximum Water Temp. (C) At Stocking
1	Rainbow	453.0	1.0	5,900	2	-
1	Cutthroat	848.2	0.75	3,180	21	-
1	Cutthroat	848.2	1.0	6,784	700	-
2	Rainbow	458.9	1.0	10,096	0	11.6
2	Cutthroat	870.0	0.75	3,915	0	11.6
3	Rainbow	453.0	1.0	5,889	20	17.2
3	Cutthroat	848.2	0.75	8,270	20	17.2

1/ All bags contained 1.2 gallons chilled water.



Test Number	Species Tested	Number Per Pound	Pounds In Bag	Gallons Water	Amount (seconds) Oxygen Added
13	Control	-	-	1.2	10
14	Control	-	-	1.2 (non-iced)	10

Figure 8. Dissolved oxygen contents and temperature change rates for control tests 13 and 14.

The fact that, in early tests, cutthroat trout were unable to be held at the same densities as rainbow trout led to the question of why this was the case. Possible explanations offerred for this finding were that since smaller fish have higher rates of metabolism, cutthroat trout probably consume more oxygen than an equal weight of larger rainbow trout, or that cutthroat trout react differently to the stress of being held in polyethylene bags than do rainbow trout of similar size. To determine if either explanation was correct, fish from the same vats as those used in earlier trials were tested again after growing and approximately doubling in size. At the time of these trials, the cutthroat trout were slightly larger than the rainbow trout in earlier tests.

Tests 3 and 4 (Fig. 5) revealed that rainbow trout twice the size of those used in earlier tests could not be held at higher densities than those in the earlier tests. For the sizes of rainbow trout tested, a loading density of 1 lb/1.2 gal appears to be the upper limit at McCall.

Tests 10 and 11, however, revealed improved performance of larger-sized cutthroat trout over smaller fish **in** earlier tests when loaded at a density of 1 lb/1.2 gal, but very poor performance when loaded at a density of 1.25 lb/1.2 gal. The fact that cutthroat and rainbow trout of similar sizes in Test 10 and Test 1, respectively, reacted similarly to loading densities of 1 lb/1.2 gal supports the hypothesis that the reason cutthroat at 1,198.6/lb could not be held at the same densities as rainbow at 560.7/lb is because of the higher rate of metabolism for smaller fish. The larger-sized cutthroat trout were not loaded at the higher densities in field applications, however, but presumably they would perform satisfactorily.

Why the larger rainbow trout in Tests 3 and 4 did not exhibit similar improved responses due to increased size is unclear. In fact, the oxygen content curves and mortalities in Tests 3 and 4 were very similar to those **in** Tests 1 and 2, even though the fish in the former test were roughly twice as large as those in the latter test. It may be that rainbow trout in this size range do not exhibit different metabolic rates, and therefore little difference in oxygen consumption would be noted in these tests.

This study confirms the fact that, at McCall Hatchery, rainbow and cutthroat trout fry of the size range tested cannot be successfully held in polyethylene bags for six hours at densities exceeding 1 lb/1.2 gal, and smaller sizes of cutthroat trout cannot exceed densities of 0.75 lb/1.2 gal. These densities are equivalent to 0.83 lb/gal and 0.63 lb/gal, respectively. However, Gebhards (1965) reports successfully holding rainbow and cutthroat trout of various size ranges in polyethylene bags successfully for periods up to 12 hours at Mackay Hatchery when loaded at a density of 0.75 lb/0.5 gal. This density is equivalent to 1.5 lb/gal and represents a density 50% greater than the maximum density sustainable at McCall Hatchery for twice the time periods.

A test using a loading density equal to that reported by Gebhards was performed (Fig. 7) and resulted in the highest sustained oxygen levels of any test during this study. Also, since no mortality was experienced in this trial, the fish tested apparently sufferred the least amount of stress of all fish in the study. An obvious benefit of using a smaller amount of water in bags is the rapidity of oxygen absorption in the water as shown in Test 12, as well as in the control Test 15 as compared to all other tests and controls. By filling the bag with a larger volume of oxygen, more oxygen is available to the fish, and presumably would sustain them for longer periods of time.

It is clear from this final test that considering lb/gal will not completely predict the performance of trout fry held in polyethylene bags, but rather some consideration of the amount of oxygen injected into each bag must also be given. Gebhards (1965) describes oxygen-to-water ratios of 3 to 1 as yielding satisfactory survival rates, but does not describe how the volume of oxygen injected in each bag was measured. In this study, only relative amounts of oxygen injected into each bag could be determined since a time release of oxygen at 75 psi was the method used. In Test 12, which yielded the best results, 20% more oxygen was injected than in previous tests. This method, however, does not describe the absolute volume of oxygen injected into each bag, and consequently, is not easily used to determine proper ratios of fish, water, and oxygen to load in polyethylene bags. In future studies, a flow meter that rapidly delivers oxygen into bags and measures oxygen passed in liters/minute or some similar units should be used.

One water quality parameter that was not considered in this study that may have a direct bearing on the maximum loading density achieved in polyethylene bags at McCall Hatchery is pH. Gebhards (1965) used water at a pH of 7.7 and achieved better results in tests similar to those reported here using water at a pH of 6.8. Proportions of toxic ammonia and carbon dioxide are directly related to pH values (Amend et al 1982), with the accumulation of toxic levels of carbon dioxide more favorable at acidic values while alkaline values favor accumulation of toxic levels of ammonia.

Gebhards (1965) reported no difference in mortality rates of trout between tests using tris buffer and tests with no buffer. He did report lowered levels of carbon dioxide in tests using the buffer, but felt higher levels of carbon dioxide were desirable and helped anesthetize the fish tested, thereby lowering metabolic rates and reducing oxygen consumption. Amend et al. (1982), however, demonstrated with tropical fish loaded in bags that lower carbon dioxide levels controlled with tris buffer and lower ammonia levels controlled with clinoptilolite resulted in lower mortality rates.

Based on these results, it would be advantageous to conduct tests at McCall Hatchery with trout loaded in bags containing water treated with tris buffer and clinoptilolite to determine if these additives will increase survival of trout loaded at densities greater than those determined to be satisfactory in this study.

RECOMMENDATIONS

1. Conduct further tests with various densities of trout held in 0.5 gal water in polyethylene bags to determine if better results will be achieved than with 1.2 gal water.

2. Develop an index relating weight of fish sustained in a volume of water in bags injected with a volume of oxygen (i.e.: lbs fish/gal H,0/liter 0) and determine maximum values for this index at McCall hatchery.

3. Investigate the use of tris buffer and clinoptilolite to control accumulations of carbon dioxide and ammonia in polyethylene bags containing trout fry.

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